

REMARKS

The present invention is directed to a method for inhibiting rejection of transplanted donor tissue by treating viable mammalian allogeneic and xenogeneic transplant tissue, for example, by enzymatic treatment, in such a manner as to **temporarily ablate** MHC Class I antigen complexes from the surface of the transplant tissue, then transplanting the treated donor tissue into a host before reappearance of MHC Class I antigen complexes occurs on said donor tissue. (See, e.g., page 3, line 30, to page 4, line 2, of the specification and Claim 1.) Ablation of MHC Class I antigen complexes on the donor tissue prevents the immediate immune recognition and attack by cytolytic T cells (CTLs) of the host (such recognition and attack being MHC Class I antigen complex-mediated processes); and thereafter, continued expression by the viable transplant tissue of MHC Class I molecules results in the gradual reappearance of MHC Class I antigen complexes on the surface of the transplant tissue cells. (That is, the ablation of the MHC Class I antigen complexes from the donor tissue is **temporary**, see page 5, line 32, to page 6, line 2.) The gradual re-expression and re-presentation at the surface of the donor cells of MHC Class I antigen complexes provides the normal mechanism for educating the host's immune system to identify the new (donor) tissue as "self" and to cause deletion of the subpopulation of natural CTLs capable of recognizing and rejecting the donor tissue. (See, e.g., page 6, lines 6-12, and Example 2, on pages 9-10 of the application.)

The Office Action of January 13, 2005 sets forth the following rejections to the claims:

- I. Claims 1, 2 and 5 are deemed to be anticipated under 35 U.S.C. §102(b) by Civin, U.S. Pat. No. 5,081,030 (hereinafter "Civin").
- II. The methods recited in Claims 1-9, 12-14, and 16-23 are deemed to be unpatentable under 35 U.S.C. §103(a) in view of the combined teachings of Civin, taken with Galati et al., *Cytometry*, 27: 77-83 (1997) (hereinafter "Galati"), Faustman & Hayashi, U.S. Pat. No. 6,617,171 (hereinafter "Faustman et al."), Lee

et al., U.S. Pat. No. 5,670,358 (hereinafter "Lee"), and Brownlee et al., U.S. Pat. No. 6,156,306 (hereinafter "Brownlee").

- III. The methods recited in Claims 1-14, and 16-23 are deemed to be unpatentable under 35 U.S.C. §103(a) in view of the combined teachings of Civin, taken with Galati, Faustman et al., Lee, and Brownlee, and further in view of Stone et al., *Transplantation*, 65: 1577-1583 (1998) (hereinafter "Stone").

Reconsideration and allowance of all claims are respectfully requested, for the reasons set forth below.

I. The Civin reference does not anticipate the methods of Applicant's claims

The Civin reference cited by the Examiner does not disclose a method for inhibiting rejection of viable donor tissue by a host comprising the steps of (a) treating the viable donor tissue with an enzyme such as papain to temporarily remove (ablate) MHC Class I surface antigens prior to transplantation, (b) utilizing the treated donor tissue for transplantation into the host prior to re-expression of new MHC Class I surface antigens by the treated donor tissue, and (c) maintaining the viable donor tissue in the host after transplantation, i.e., such that MHC Class I surface antigens are re-expressed on the surface of said donor tissue *in situ*, thereby reeducating the host immune system to recognize and accept the donor's MHC Class I surface antigens as self molecules.

Critically, the Civin reference especially does not teach step (b) of Applicant's inventive method. Civin only discloses a method for sorting cells using monoclonal antibodies immobilized on a chromatographic substrate which recognize a particular cell surface epitope that characterizes the desired cells. In the case of Civin, anti-CD34 antibodies (recognizing the MY10 epitope of CD34) are immobilized on magnetic beads, thus targeting the beads to CD34-positive cells. After the magnetic bead-bound CD34-positive cells are separated from non-CD34-positive cells (i.e., mature B cells, T cells, NK cells, monocytes, granulocytes, erythrocytes, platelets), the CD34-positive cells are released intact from the magnetic beads by enzymatic treatment (e.g., papain,

chymopapain) to cleave the anti-CD34 antibody which links the cells and magnetic beads together.

The Civin reference does not teach treatment of any donor cells to temporarily ablate MHC Class I surface antigens by enzymatic treatment prior to transplantation. Nor does the reference relate at all to induction of tolerance to viable transplant tissue by maintaining a MHC Class I-ablated transplant tissue in the host recipient until re-expression of MHC Class I antigens occurs in the transplant tissue after transplantation, and the host accepts the donor's MHC Class I surface antigens as "self".

The Examiner asserts that

"[Civin] teaches a method for transplantation of donor tissue cells that are enzymatically treated in order to remove surface molecules or glycoproteins antigens..." (Office Action at page 4),

however this is blatantly incorrect: The CD34-positive stem cells separated by the chromatographic technique of Civin are useful for so-called bone marrow transplantation, however the cells themselves are not treated in any way to better prepare them for receipt by a host. The enzymatic treatment described in Civin is used merely as a means of breaking the extracellular cross-link to an affinity matrix. This can be understood simply by reading the title of the patent. More important, it is not a teaching of Civin that any surface structure of the CD34-positive cells is disturbed or ablated in order to render the cell more compatible with a particular host -- such a treatment is not contemplated by the inventors of Civin and is certainly not disclosed by the reference. Indeed, the fact that the cells and their essential properties are unchanged is emphasized in Civin:

"Reproducible enzymatic cleaving of immunomagnetic microspheres from MY10-positive cells can be achieved by brief treatment of the preparation with papain or chymopapain. The chymopapain **treatment does not produce detectable damage to human colony-forming cells or rat stem cells.**" (U.S. 5,081,030 at col. 3, lines 52-57; emphasis added.)

The Examiner comments that the Civin reference is considered to disclose "identical active steps" to the method of the invention, however this is not the case:

With regard to the enzymatic treatment step (a) of Applicant's claimed method, it is clear that Civin teaches enzymatic treatment only to release isolated cells from an affinity matrix, whereas Applicant teaches enzymatic treatment specifically to cause an immunologically significant change in the cell surface of the tissues intended for transplant. It is pointed out that the CD34-positive cells addressed in Civin are immature bone marrow cells, which do not exhibit the same cell surface immunological markers as mature cells. Whether the enzymatic exposure necessary to release such cells from an affinity matrix is equivalent to enzymatic treatment of cells to temporarily ablate MHC Class I antigens from the cell surface is a matter of conjecture for the Examiner. Since there is no direct teaching in Civin to temporarily remove MHC Class I antigen complexes from the surface of CD34-positive cells, and since the Civin reference to the contrary teaches that it is an advantage that the enzymatic release of the cells "does not produce detectable damage" to the cells, a fairer interpretation of Civin is that it teaches away from use of papain or chymopapain to an extent that will change the immunological characteristics of the cells, as is called for expressly in step (a) of the Applicant's claims. In other words, with regard to the enzymatic treatment, the Civin reference does not teach the use of enzymes sufficient to remove immunogenic surface structures but rather only teaches enzymatic treatment sufficient to release cells from a matrix while leaving cell properties intact. Such teaching is not "identical" or equivalent to step (a) of Applicant's method.

Regardless of what is the inherent result of the enzymatic release from a separation matrix taught by Civin, there is absolutely no teaching of step (b) of Applicant's invention contained in the Civin reference. That is, Civin does not teach or even contemplate the active step of using viable, enzymatically treated tissues (temporarily ablated of MHC Class I antigens) for transplantation into a host mammal *before MHC Class I antigens are re-expressed on the surface of the donor tissue*, as is expressly required by Applicant's method. Because Civin does not contemplate ablation of MHC Class I antigens from cells as a positive step, there is no way for Civin to be interpreted as teaching use of the treated cells before such ablated antigens are re-expressed. This is a critical, recited feature of Applicant's method which is not within the

teaching of Civin, ergo Civin cannot be proposed to anticipate the present invention as claimed.

Because the Civin reference is incapable of teaching critical recited steps of Applicant's claims, the Civin reference does not anticipate Claims 1, 2, and 5 under 35 U.S.C. §102(b), and withdrawal of this rejection is in order.

II. The combination of Civin taken with Galati, Faustman et al., Lee, and Brownlee does not render Claims 1-9, 12-14 and 16-23 obvious under 35 U.S.C. §103(a)

In the Office Action, the Examiner has rejected Claims 1-9, 12-14, and 16-23 under 35 U.S.C. §103(a) as being unpatentable over Civin, taken with Galati, Faustman et al., Lee, and Brownlee.

Reconsideration is respectfully requested. The multi-reference combination of Civin taken with Galati, Faustman et al., Lee, and Brownlee fails to present a suggestion of the methods of Claims 1-9, 12-14, and 16-23 of Applicant's invention.

First of all, the Faustman et al. reference, U.S. 6,617,171, must be removed from the combination of references because it is not a description of prior art to the present invention. Submitted herewith is the declaration of Denise L. Faustman, the inventor herein, who identifies the descriptions of U.S. 6,617,171 relied on by the Examiner as descriptions of her own work. Thus, the Faustman et al. patent does not represent a disclosure in an application "by another" and does not represent a description of work occurring "before the invention by the applicant". See, *In re Katz*, 687 F.2d 450, 215 USPQ 14 (CCPA 1982); MPEP §715.01(c).

For the reasons set forth above, the teachings of Civin can be seen to be unrelated to the novel methods taught in Applicant's application and, as discussed below, the Galati, Lee, and/or Brownlee references do not provide sufficient disclosure to link the teachings of Civin with the present invention.

The concept of attempting to inhibit transplant rejection by a host by temporarily ablating the MHC Class I antigens presented on the surface of donor tissue prior to transplantation, then transplanting the MHC Class I antigen-ablated donor tissue prior to re-expression of MHC Class I antigens, is nowhere found in the cited prior art. As exhaustively reviewed above, the Civin reference only includes teachings related to

releasing cells immobilized on a chromatographic matrix. The importance of MHC Class I antigens to transplant rejection is not discussed in Civin, and consequently the importance of introducing a transplant which avoids exposure of the host to such surface antigens of the donor tissue cannot be taught by Civin, either, although it is a critical requirement of Applicant's claims.

The secondary Galati reference does not even relate to the field of transplantation. Rather, Galati discloses an *in vitro* method for MHC Class I antigen expression involving the preparatory step of removing intact MHC Class I antigen complexes from living cells, then collecting the complexes and measuring an acid-isolated component thereof (β_2 -microglobulin) as a means of quantitating the expression level of Class I antigen complexes on the original cells. No reference to transplantation of living cells is made in Galati; it is a document relating to laboratory methods.

The Lee reference actually teaches away from treating cells with papain or chymopapain. Lee discloses a method for digesting tissue, in particular connective tissue with papain or chymopapain to dissolve the tissue and release living cells from the connective tissue. More specifically, Lee teaches a method for inhibiting the enzymatic effect of the digestive enzymes papain and chymopapain:

"An exemplary process of the present invention includes enzymatically digesting connective tissue by providing an enzyme composition containing papain or chymopapain, or a mixture of papain and chymopapain, in an amount sufficient to hydrolyze connective tissue and dissociate desired viable cells from such tissue . . . **It is essential to halt or at least substantially slow down the enzymatic activity in the medium containing the isolated viable cells as soon as possible after the cells are dissociated from the tissue in order to preserve the cell integrity.** This is accomplished by preventing excessive digestion . . . Following addition of an inhibitor in accordance with the present invention, the viability of the isolated cells is greatly preserved and the yield of viable cells is increased." (Lee at col. 2, lines 21-38; emphasis added.)

Therefore, Lee teaches that once the connective tissue has been digested to the point of release of the cells, the reaction should immediately be stopped to prevent enzymatic damage to the isolated cells. No association of enzymatic digestion with immunologically augmenting the cell to be more suitable for transplant is made in Lee. Moreover, no reference as to re-expression of MHC Class I antigens is made in Lee.

Brownlee teaches a method for inhibiting MHC Class I antigen presentation permanently in cells as a means of avoiding transplant rejection based on immune recognition of intact MHC Class I antigens. The method is based on the observation that virally infected cells are sometimes able to avoid immune attack by upsetting normal MHC Class I antigen presentation.

"The invention provides a method and vectors to express a gene, derived from a virus, that blocks the intracellular transport and/or intracellular maturation within the graft of proteins called MHC class I products." (Brownlee at col. 5, lines 31-35.)

Brownlee does not teach or disclose the treatment of donor tissue with an enzyme to temporarily ablate the expression of MHC Class I prior to transplantation to prevent host rejection. There is also no suggestion in Brownlee to make use of enzymatically treated cells in transplantation prior to re-expression of normal MHC Class I molecules on the treated donor cells -- the treated cells according to Brownlee have lost the ability to normally express MHC Class I antigens.

The Examiner, at page 6 of the Office Action, relies on the Brownlee reference for a property of control, untreated cells used for comparison to cells according to the Brownlee teaching:

"Stocks of replication defective virus are prepared according to the teaching of WO 92/07945. The effects of pHSVgp19 are tested as follows. A human embryonic kidney cell line (293) is plated at about 5×10^5 per 35 mm diameter well and cultured for 24 hours at which time a concentrated stock of HSV amplicon pHSVgp19 is added to the culture to achieve a multiplicity of infection of about 2. After 5 hours the cells are washed and continued in culture another 19 hours.

The cells are then trypsinized and removed from the culture well, washed and treated with 10 mg/ml papain in a 0.01 M pH 7.5 Tris buffered normal saline, 0.01 M cysteine for 45 min. The cells are washed and replated and cultured until control cultures which were papain treated but not transfected with pHSVgp19 have reexpressed MHC class I products. The cultures are then prepared for FACS analysis." (Brownlee at col. 16, lines 1-17.)

It is noted that this example from Brownlee appears to be a prophetic example rather than an actual example; therefore, its usefulness as a "teaching" of Brownlee as opposed to speculation (which might be discounted by a person skilled in the art) is questioned. Nevertheless, the preparation of a control culture having the ability to re-express MHC Class I molecules after papain treatment does not amount to a teaching to use such control culture as transplant tissue -- especially in a disclosure devoted to teaching the use of the permanently altered, non-MHC Class I producing experimental culture. Furthermore, even recognizing that the non-inventive control culture re-expresses MHC Class I products according to Brownlee, there is no suggestion to use such cells for transplant PRIOR TO said re-expression occurring. This is the feature which is expressly recited as step (b) of Applicant's methods but which is totally absent from Brownlee and all the other references cited in rejecting the claims.

Thus, the combined disclosures of Civin taken with Galati, Lee, and Brownlee do not have what it takes to suggest the method of the present invention as claimed. Accordingly, reconsideration and withdrawal of the rejection based on 35 U.S.C. §103(a) are respectfully solicited.

III. The combination of Civin taken with Galati, Lee, Brownlee and Stone does not render Claims 1-14 and 16-23 obvious under 35 U.S.C. §103(a)

In the Office Action, page 7, the Examiner has rejected Claims 1-14 and 16-23 under 35 U.S.C. §103(a) as being unpatentable over the combination of Civin, taken with Galati, Faustman et al., Lee and Brownlee, further in view of Stone. The Stone reference is relied on to supplement the Examiner's combination applied against Claims 1-9, 12-14, and 16-23 with a disclosure relevant to the α -galactosidase features of Claims 10 and 11.

For the reasons set forth above in subsection II, the combination of Civin taken with Galati and further in view of Faustman, Lee and Brownlee, does not render obvious the present invention as set forth in Claims 1-9, 12-14, and 16-23. Applicant further asserts that the combination of these references with the Stone reference does not give a teaching to render obvious the subject matter of Claims 1-14 and 16-23.

Claims 10 and 11 depend from Claim 8 (specifying treatment of the donor tissue with a second enzyme) which, in turn, depends from Claim 1 (specifying, *inter alia*, treatment of the donor tissue with an enzyme effective for temporary ablation of MHC Class I antigens from the tissue). Claim 10 specifies that the second enzyme is α -galactosidase; Claim 11 specifies that the donor tissue is treated with a combination of papain and α -galactosidase.

As demonstrated in detail above, the Civin patent, taken with the Galati, Lee, and/or Brownlee references, would not have suggested to a person of ordinary skill in the art at the time of Applicant's invention a method for inhibiting rejection of viable transplant tissue by (a) temporarily ablating MHC Class I antigens from the surface of said viable donor tissue then (b) transplanting said tissue before re-expression of MHC Class I antigens by the donor tissue occurs. The inclusion of Stone as an additional reference does not improve this combination and in fact renders the combination *less* capable of suggesting Applicant's invention, by providing additional teachings *away* from the use of viable donor tissue for transplantation.

With respect to Stone, the Examiner states,

"[Stone] discloses a method for inhibiting transplant [rejection] wherein the method comprises [the] step of treating donor tissue with galactosidase and [the] step of transplanting the treated tissue into host recipient and wherein the method results in a reduction of inflammatory reaction or immune response of recipient host." (Office Action, page 8.)

The Stone reference reports on the effect of eliminating α -Gal epitopes from porcine articular cartilage, by incubation with α -galactosidase followed by implantation into the suprapatellar pouch of cynomolgus monkeys and monitoring of the immune response. (*See*, Stone, at page 1578, right column, 1st paragraph.)

According to Stone,

"This study shows that treatment of cartilage with α -galactosidase can successfully prevent anti-Gal immune response against the xenograft. However, the primate immune system reacts against cartilage-specific antigens, resulting in antibody formation as well as macrophage-mediated chronic inflammatory reaction in some of the xenografts." (Stone at page 1578, right column, 1st paragraph.)

Stone teaches the use of cartilagenous tissue which clearly still exhibits porcine antigens that cause immune attack of the transplanted tissue by the host immune system. There is no teaching relating to the viability of the transplant tissue and no suggestion that the transplanted tissue retains the ability to re-express any normal antigens removed by enzymatic treatment.

Indeed, as taught by the Stone reference, the harvested cartilage tissue is prepared for transplant by first immersing in alcohol for 5 minutes followed by immersion in a phosphate-citrate-sodium-chloride buffer containing 100 U/ml α -galactosidase for 4 hours at 26°C. (See, Stone, Materials and Methods, page 1578.) Applicant asserts that it would be appreciated by a person of ordinary skill in the art that such treatment with alcohol would be lethal to the transplant tissue; accordingly, the Stone reference relates to the use of non-viable tissue for transplantation. Under the circumstances, the Stone teaching cannot supplement the basic reference combination of Civin in view of Galati, Lee and Brownlee (Faustman et al. not being prior art) to provide any suggestion of the Applicant's inventive methods.

For the foregoing reasons, Applicant's invention as recited in Claims 1-14 and 16-23 is not obvious in view of the combination of references asserted by the Examiner, and therefore reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) are respectfully requested.

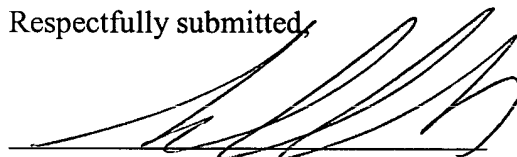
CONCLUSION

Applicant respectfully submits that all rejections of the Office Action dated January 13, 2005 should be reconsidered and withdrawn, for the following reasons:

- The rejection based on 35 U.S.C. §102(b) is incorrect, as the cited Civin reference does not teach each and every element of Applicant's claimed invention. In particular, the Civin reference relates only to a method for releasing immature CD34-positive cells from a chromatographic matrix, and it fails to teach the use of enzymatically treated but viable donor tissue for transplantation prior to the re-emergence of MHC Class I antigens on the donor tissue.
- The two reference combinations relied on by the Examiner to make rejections under 35 U.S.C. §103(a) fail to establish the obviousness of any of Claims 1-14 or 16-23 because neither reference combination suggests the concept of (a) temporary ablation of an antigenic structure from donor tissue prior to transplantation, followed by (b) use of the ablated donor tissue for transplantation before the ablated antigenic structure is re-expressed.

Accordingly, for the reasons set forth herein, reconsideration and allowance of Claims 1-14 and 16-23 are respectfully solicited.

Respectfully submitted,

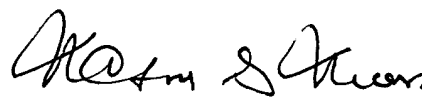


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